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A prospective validation pharmacogenomic study in the adjuvant setting of colorectal cancer patients treated with the 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX4) regimen

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Abstract

The discovery of pharmacogenomic markers in colorectal cancer (CRC) could be setting-specific. FOLFOX4 is employed in the adjuvant and metastatic setting in CRC. This prospective study is aimed to validate in the adjuvant setting the pharmacogenomic markers of toxicity reported in the metastatic setting (that is, GSTP1-rs947894, and -rs1138272; GSTM1-null genotype; AGXT-rs4426527, -rs34116584 and del-74 bp), and to discover additional markers. CRC patients (n = 144) treated with adjuvant FOLFOX4 were genotyped for 57 polymorphisms in 29 genes. Grade ≥2 neurotoxicity was associated false discovery rate-adjusted q-value <0.1) with single-nucleotide polymorphisms in *ABCC1* (rs2074087: odds ratio = 0.43(0.22–0.86)), and *ABCC2* (rs3740066: 2.99(1.16–7.70); rs1885301: 3.06(1.35–6.92); rs4148396: 4.69(1.60–13.74); rs717620: 14.39(1.63–127.02)). hMSH6-rs3136228 was associated with grade 3–4 neutropenia (3.23(1.38–7.57), q-value = 0.0937). *XRCC3*-rs1799794 was associated with grade 3–4 non-hematological toxicity (8.90(2.48–31.97), q-value = 0.0150). The markers previously identified in metastatic CRC were not validated. We have identified new markers of toxicity in genes of transport and DNA repair. If validated in other studies, they could help to identify patients at risk of toxicity.

Keywords

pharmacogenomics; FOLFOX4; colorectal cancer; neurotoxicity

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INTRODUCTION

Validated markers have been recently identified for treatment personalization in oncology, with drug toxicity as the outcome of interest.[1,2] This appears to be a promising field for improving the management of colorectal cancer (CRC) therapy. However, most of the data have been generated in the metastatic setting[3–6] and it is not clear whether these markers could be applied in different clinical settings in CRC.

Oxaliplatin in combination with fluoropyrimidines (FOLFOX4 schedule) is largely used in the treatment of CRC in both the metastatic and the adjuvant settings. However, oxaliplatin-based chemotherapy is limited by the occurrence of severe toxicities in a considerable number of patients.[7–9] Among those side effects, peripheral neurotoxicity is one of the most common and disabling. Increasing cumulative doses of oxaliplatin lead to a persistent sensory peripheral neuropathy associated with functional disability in some patients.[10,11] Recent investigations establish the persistence of subjective and objective deficits in oxaliplatin-treated patients after the interruption of oxaliplatin, suggesting that sensory neuropathy could be a long-term sequela in more sensitive patients thereby challenging the notion of its reversibility.[12] Moreover, clinical studies investigating oxaliplatin neurotoxicity seem to indicate a synergistic neurotoxic effect with fluoropyrimidines.[13]

In vitro and *in vivo* studies on neuronal damage mechanisms[14] indicate that oxaliplatin exerts a direct pharmacological effect on the excitability of sensory neurons and muscle cells by interfering with voltage-gated Na+ channels.[15] Another suggested mechanism is the deposition of DNA-platinum adducts in the dorsal root ganglions, inducing neuronal damage and apoptosis,[16] with the involvement of the glutathione-related detoxification system and the DNA repair protein complex.[17]

Germline variants in genes encoding DNA repair enzymes, detoxification pathways and ions channels may be responsible of the inter-subject variability in the occurrence of oxaliplatin toxicity. Some markers of toxicity have been previously identified in metastatic CRC patients,[18,19] but they are likely to have a different impact when dealing with adjuvant patients, especially in relation to the neurological toxicity. First, a metastatic patient could have undergone previous treatments with drugs with neurotoxic potential. In addition, tumor progression and its dissemination have been related to changes in the levels of inflammatory mediators, with associated increased oxidative stress.[20,21] This could make a metastatic patient particularly sensitive to environmental stress factors like hypoxia and oxidative stress, resulting into DNA damage upon exposure to a cytotoxic agent.[22]

To our knowledge, no data are available in the adjuvant setting on genetic polymorphisms as toxicity predictors for CRC patients treated with oxaliplatin (FOLFOX4). Hence, the primary aims of this prospective study are (1) to investigate the role of genetic polymorphisms previously identified in the metastatic disease as predictive markers of toxicity (mainly neurotoxicity and neutropenia) in patients treated with adjuvant FOLFOX4; and (2) to discover associations with putative novel biomarkers.

MATERIALS AND METHODS

Patients and study design

This multi-institution study was sponsored by the CRO-National Cancer Center of Aviano, Italy. CRC patients (n = 154) were genotyped for 57 genetic polymorphisms in 29 candidate genes. The main endpoint was neurotoxicity during FOLFOX4 therapy in curatively-resected patients with stage II–III CRC. Neutropenia and any non-hematological toxicity were evaluated as secondary endpoints. The highest grade of toxicity recorded during the

treatment for each toxicity endpoint (that is, neurotoxicity, neutropenia and any non-hematological toxicity) was used for association with polymorphisms. The Institutional Review Board of each participating institution approved the study protocol, and all patients signed a written informed consent before entering the study.

Patients with histologically confirmed CRC, and radiologically confirmed absence of distant metastases were eligible. Eligibility criteria were as follows: stage II–III CRC; age >18 years; performance status (WHO) 0–2; normal bone marrow, renal and liver function. Patients affected by chronic inflammatory enteric diseases, evidence of neurosensory disease or assuming neurotoxic medications were excluded from the study. All the patients were of Caucasian ethnicity and have been enrolled in centers located in northern and Central Italy.

Eligible patients were treated with FOLFOX4 (oxaliplatin 85 mg m^{-2} (2 h infusion on day 1), leucovorin (100 mg m^{-2} as 2 h infusion on day 1), 5-fluorouracil bolus (400 mg m^{-2}) and 22 h infusion (600 mg m^{-2}) on days 1 and 2 every 2 weeks) for 6 months (12 cycles).

Toxicity evaluation

Objective clinical evaluation, blood counts, hepatic and renal function tests were performed within 48 h before each cycle. Patients were questioned about nausea and vomiting, mucositis, diarrhea, asthenia (that is, fatigue, malaise and weakness symptoms), and appetite at every cycle. Toxicity was evaluated according to NCI-CTC criteria version 2.0 (http://ctep.cancer.gov/). Neurotoxicity was evaluated according to the oxaliplatin-specific scale.[23] Patients undergoing at least one cycle of chemotherapy were included in this study.

Chemotherapy was delayed until recovery from hematological toxicities or in the case of significant, persisting, non-hematological toxicity. In the event of severe (grade 3–4) toxicity, the doses of oxaliplatin and 5-fluorouracil were reduced by 25 or 50% based on the physician's evaluation. Treatment was discontinued either in the event of anaphylactic reaction, or repeated severe toxicity in spite of dose reduction, or patient refusal.

Selection of candidate polymorphisms and genotyping assay

Genomic DNA was extracted from peripheral blood using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). Positive controls were included in the analyses (that is, for each polymorphism, three DNA samples for each genotype, determined by an alternative genotyping procedure, were used as reference). Genotyping was performed blinded to the clinical data.

Two different criteria were used for the selection of candidate genes for the pharmacogenomic analysis: (1) gene variants included in previous pharmacogenomic association studies of FOLFOX in metastatic CRC.[18,19,24–27] (2) Variants in genes in cellular pathways known to be involved in the pharmacokinetics and pharmacodynamics of oxaliplatin (www.pharmgkb.org). Genes, variants, pathways and assays are listed in Supplementary Table 1.

For pyrosequencing we used PSQ96MA (Biotage, Uppsala, Sweden). PCR amplifications were performed in an Eppendorf Mastercycler gradient, with TaqGold DNA Polymerase (AB Applied Biosystems, Warrington, UK). Detailed Pyrosequencing genotyping protocols are available upon request. Predesigned or custom Taq Man single-nucleotide polymorphism (SNP) genotyping assays were used for the allelic discrimination reactions. Predesigned Taq Man Copy Number assays were used on ABI 7900HT (AB Applied Biosystems, Foster City, CA, USA) for the analysis of gene deletion polymorphisms. All the commercial Taq Man assays were purchased from Applied Biosystems (www.appliedbiosystems.com). Analyses with Taq Man assays were performed with the Applera Taq Man Universal Master mix on

ABI 7900HT (AB Applied Biosystems) according to the manufacturer instructions. Automated fragment analysis was performed on ABI Prism 3130 (Applied Biosystems), with Gene Scan software (Applied Biosystems). Detailed fragment analysis and gel electrophoresis genotyping protocols are available upon request. *AGXT* polymorphisms were analyzed as previously reported.[25]

Statistical analysis

The study was prospectively designed to test the association between genetic polymorphisms and oxaliplatin neurological toxicity (grade ≥2), as the primary endpoint. Severe neutropenia and any severe non-hematological toxicity were evaluated as secondary endpoints. For each polymorphism, deviation from Hardy–Weinberg equilibrium was tested by Fisher's exact test and no deviation was found ($P>0.05$). Odds ratio and 95% confidence interval were estimated by unconditional logistic regression. We investigated three genetic models (that is, dominant, recessive and additive) for the association, and the most statistically significant by Wald χ^2-test was reported. All P values were two-sided. Logistic models adjusting for age and sex of the patients, neo-adjuvant treatment (yes/no) and cumulative oxaliplatin dose normalized by body surface area (mg m^{-2}), were run. The potential confounding effect of pre-operative treatment was also tested through a sensitivity analysis by excluding patients who underwent pre-operative radio- or chemotherapy. The SAS software (version 9.2) was used for all the analyses.

To control for multiple testing, q-value (a false discovery rate (FDR)-adjusted P value, FDR 0.1) was calculated for each SNP implemented in the R-package.[28]

During the review process of this paper, a *post-hoc* power analysis was performed for the main study endpoint, that is, severe neurotoxicity, using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).[29] This study has a power of 79.2, 57.8 and 30.2% to detect a genotype relative risk of 1.5 with an alpha-level of 0.05, 0.01 and 0.001, respectively. The power was evaluated under the assumption of the following parameters: additive inheritance model, prevalence of severe neurotoxicity phenotype = 0.4 and average risk allele frequency = 0.30.

Pairwise linkage disequilibrium (LD) analysis for *ABCC1* and *ABCC2* polymorphisms was performed by HaploView 3.32.[30] Haplotypes and diplotypes were inferred by Phase version 2.1.1.[31,32]

RESULTS

Patient characteristics

Ten of the 154 patients enrolled into the study were considered ineligible for this pharmacogenomic analysis according to the study criteria (1 was stage I; 2 were completely resected stage IV; 1 received 5-fluorouracil alone; 1 received FOLFOX plus bevacizumab, 5 were lost during follow-up), thus leaving 144 eligible patients. The majority of the patients (56%) completed all the 12 planned cycles of chemotherapy (mean of 10 cycles, range 1–12). Nineteen patients (13%) underwent neo-adjuvant radiotherapy with or without systemic chemotherapy (Table 1).

Grade 3–4 toxicity of any type was experienced by 48% of patients (69/144): 18% (26/144) developed non-hematological grade 3–4 toxicity and 39% (56/144) developed hematological grade 3–4 toxicity. Neutropenia (38%) was the most common severe hematological toxicity, whereas diarrhea (11%) and neurotoxicity (7%) were the most frequent non-hematological severe toxicities. Neurotoxicity was also the most common adverse event of any grade

developed during the treatment (83%). A detailed description of patient toxicity is in Table 2.

Validation of polymorphisms previously associated with FOLFOX4 toxicity in the metastatic setting

We included in the analysis 23 genetic polymorphisms previously investigated in metastatic CRC patients treated with FOLFOX4. Six of them were associated with severe toxicity (either neurotoxicity or neutropenia) in at least one study. GSTP1-rs947894 and rs1138272, AGXT-rs4426527, rs34116584 and del-74 bp were associated with neurological toxicity, whereas GSTM1-null genotype was related to severe neutropenia (Supplementary Table 1). None of the associations reported in previous studies in the metastatic setting were replicated in the present study (Table 3).

New discoveries of genetic polymorphisms and neurological toxicity

Neurotoxicity of grade ≥2 was the primary endpoint for this analysis, because of its prevalence (39%) and clinical relevance (grade 2 indicates moderate motor symptoms and sensory symptoms extended to ankle and wrist, and grade 3 indicates motor symptoms requiring help/assistance and sensory symptoms extended to knee and elbow).

Polymorphisms in *ABCC1* and *ABCC2* were associated with this phenotype (Table 4). The minor frequency alleles of rs2074087 and rs35587 in *ABCC1* were associated with a reduced risk of neurotoxicity. The minor frequency alleles of rs3740066, rs1885301, rs4148396 and rs717620 in *ABCC2* were associated with an increased risk of neurotoxicity, whereas the minor frequency allele of rs2273697 was associated with a reduced risk of neurotoxicity. The minor frequency allele of rs2622604, in *ABCG2* was associated with an increased risk of neurotoxicity. FDR analysis pointed out that five out of eight predictive markers have a *q*-value <0.1.

Analysis of LD among the above SNPs identified significant LD among the *ABCC2* polymorphisms (Figure 1), but not among the *ABCC1* polymorphisms (results not shown). Therefore, we performed a haplotype analysis on *ABCC2* variants to test whether haplotypes are more predictive than single variants. Haplotype I (16%, all the protective alleles) and II (19%, all the risk alleles) were selected but no significant association with grade ≥2 neurotoxicity was found (Table 4).

New discoveries of genetic polymorphisms and severe (grade ≥3) toxicity

Grade ≥3 neutropenia was significantly associated to polymorphisms in *hMSH6* (rs3136228), *ABCC1* (rs35587) and *ABCC2* (rs717620). One of them was below the FDR threshold. Polymorphisms in *XRCC3* (rs1799794, rs861539), *XRCC1* (rs3213239), *APE1* (rs1130409), *PARP* (rs1136410) and the *GSTT1* null genotype were associated with severe non-hematological toxicity, but only *XRCC3*-rs1799794 passed the FDR cutoff and was associated with an increased risk of toxicity (Table 5).

For any of the above associations, the exclusion of 19 pre-treated rectal cancer patients, did not result in a substantial modification of the statistical meaning of the results (results not shown).

DISCUSSION

To our knowledge, this is the first study investigating pharmacogenomic markers of toxicity in a group of CRC patients homogeneously treated with adjuvant FOLFOX4, whereas several studies have been published in the advanced disease.[18,19,26,33] There is a great

clinical interest in the discovery of predictive biomarkers of treatment outcome and toxicity in the oncological field and important results have been obtained, especially for the gastrointestinal disease.[1,34]

The introduction of FOLFOX4 has led to significant improvements in the clinical outcome of CRC patients.[7,35] FOLFOX4 has an acceptable toxicity profile, with neutropenia and sensory peripheral neuropathy as the most clinically significant and often dose-limiting toxicities. Nonetheless, the persistence of neurological toxicity after the interruption of FOLFOX4 has been recently reported in a high percentage of patients (58–83%).[12] Symptoms of neurotoxicity could persist also in patients who experienced grade 2 toxicity during the course of treatment. Therefore, there is an urgent need to identify predictive markers of mild-to-severe toxicity in order to avoid long-term disability, especially in patients with favorable prognosis. In agreement with similar previous studies,[7] 39% of patients developed grade 2 or 3 toxicity in our study. This toxicity is common and clinically relevant, and deserves a thorough investigation of its potential genetic basis. We aimed to validate previous associations in the metastatic setting, and discover new putative marker for further investigation.

A comprehensive panel of SNPs was selected to include all the most relevant polymorphisms previously reported as significant markers of toxicity in FOLFOX4 in metastatic CRC patients (Supplementary Table 1). In the present study we did not replicate the predictive role of the *GSTP1*-rs947894 polymorphism on neurotoxicity previously reported in advanced CRC patients treated with FOLFOX4.[18,19,24,26] The clinical validity of this marker has been already put into question.[25,27,36] Moreover, our study seems to suggest that its effect (if any) might be context dependent. GSTP1 is an enzyme mediating glutathione-related detoxification of oxaliplatin. This detoxification pathway might be dependent upon systemic oxidative stress, which is known to be impaired in patients with metastatic disease.[22]

Findings from similar pharmacogenomic studies conducted in different settings should be interpreted with caution. The biological scenario of patients with early disease treated with adjuvant oxaliplatin differs from that of patients with metastatic disease. The molecular and metabolic changes related to tumor metastatization involve several pathways, including the glycolysis, the tricarboxylic acid cycle, the pentose phosphate pathway, fatty acid and nucleotide biosynthesis and the GSH-dependent antioxidative pathway.[37] Thus, because of the role played by these pathways in the pathophysiology of oxaliplatin neurotoxicity,[12,38] it is plausible that genetic polymorphisms could have a different impact depending upon the clinical setting.

The panel of SNPs for genotyping in this study was enriched with SNPs of oxaliplatin pharmacology, in addition to the variants previously proposed as markers in the metastatic setting. The main finding of this study is the involvement of trans-cellular transporters, in particular ABCC2 and ABCC1, in the occurrence of FOLFOX4 toxicity. We identified five genetic polymorphisms in *ABCC2* (rs3740066, rs1885301, rs4148396, rs717620) and *ABCC1* (rs2074087) as predictors of grade 2–3 neurological toxicity, with an FDR <10%, and therefore considered noteworthy predictive markers. Similar associations were also obtained for severe neutropenia though they did not pass the 10% FDR cutoff (Table 5).

ABCC1 and ABCC2 belong to ATP-binding cassette transporter superfamily, containing several family members that mediate the cellular trafficking of drugs, their metabolites and endogenous factors.[39] ABCC1 and ABCC2 may act in a synergistic way modulating the effect of oxaliplatin and 5-fluorouracil at the cellular level.[40]

ABCC2-rs717620, located on the gene promoter region, has been previously associated to decreased protein expression *in vitro*,[41] and was associated with a 13-fold increased risk of grade 2–3 neurological toxicity in our study. It was also associated to a five-fold increased risk of severe neutropenia, though this association had a *q*-value higher than 0.10 (0.1228) (Table 5). *ABCC2*-rs717620 and rs3740066 had a combined effect in increasing platinum-related toxicity in lung cancer patients,[42] similar to our results. In addition to the ABCC2 variants passing the FDR cutoff, ABCC2-rs2273697 was associated with a protective effect for grade 2–3 neurological toxicity in our study (Table 4). Although this association did not pass the FDR cutoff (*q*-value 0.1109), rs2273697 is a missense polymorphism causing a Val417Ile amino-acid substitution increasing the transporter efficiency.[41] Enhanced ABCC2 expression can lead to decreased cellular glutathione content.[43] Glutathione is needed for oxaliplatin detoxification via conjugation, and it was reported that low glutathione intra-cellular levels can cause increased oxaliplatin cytotoxicity.[40] Moreover, ABCC2 mediates the export of the oxaliplatin-glutathione conjugated form, and ABCC2 overexpressing cells were resistant to platinum derivatives.[44] Taken together with our results, *ABCC2* variants might change the susceptibility of patients to oxaliplatin toxicity via a glutathione-mediated mechanism.

For *ABCC1*, three genetic polymorphisms were associated with grade 2–3 neurological toxicity and one of them was also associated to severe neutropenia. The functional effect of these variants is not known, and they are not in LD with any known *ABCC1* functional variants. Overexpression of the ABCC1 protein was related to resistance to 5-fluorouracil *in vitro*.[40] This could be due to the ability of ABCC1 to extrude folates and thus depleting their intra-cellular availability for the activity of 5-fluorouracil. This might in part explain the effect of *ABCC1*-rs35587 on both neutropenia and neurological toxicity, suggesting that *ABCC1*-rs35587 might increase the function or expression of the ABCC1 transporter. More confirmatory studies (both at the clinical and molecular level) should be conducted to confirm the clinical associations and their mechanistic basis.

XRCC3-rs1799794 was associated with severe non-hematological toxicity (*q*-value = 0.0235). XRCC3 is a DNA repair protein that is part of the double strand break repair machinery. Its reduced activity is associated with significantly higher levels of bulky DNA adducts.[45] Our study suggests that this SNP might confer reduced DNA repair of oxaliplatin DNA adducts, leading to more toxicity. However, the molecular function of this SNP is presently unknown, and controversial data have been reported on the possible clinical role of this SNP on the response and toxicity to DNA damaging agents.[46,47]

hMSH6-rs3136228 was associated with increased risk of severe neutropenia (*q*-value = 0.0937). hMSH6, expressed in normal marrow cells,[48] deals with the DNA mis-match repair. In particular, it forms an heterodimeric complex with MSH2 able to recognize mispaired bases in DNA. A functional mis-match repair system is required for the detection of damaged DNA created by platinum derivatives.[49] The rs3136228 polymorphism, seems to modulate gene transcription causing the loss of a Sp1-binding site.[50] It is likely that the rs3136228 variant can affect mis-match repair activity in non cancer cells modulating the toxic effects of FOLFOX.

For the novel SNPs indentified in this study, the limited number of patients and lack of an independent validation cohort make our findings preliminary, requiring further confirmation. The use of an oxaliplatin-specific scale[23] could impair the ability to replicate our findings in studies using the definitions of the NCI-CTC.[51,52] Our study did not validate existing markers previously identified in metastatic patients, and provides the basis for testing the validity of new markers in future studies in adjuvant FOLFOX. Among those, the variants in

ABCC2 have a clinical effect that is consistent with their molecular function, and should be prioritized for testing of their clinical validity in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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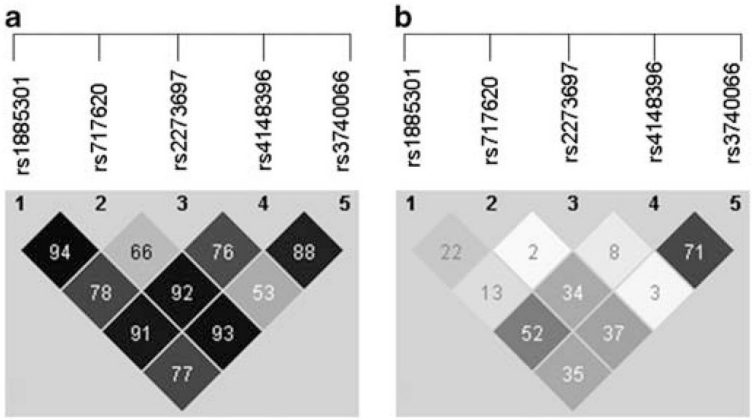

Figure 1.

Pairwise linkage disequilibrium analysis among *ABCC2* polymorphisms associated to neurotoxicity grade ≥2. The value within each diamond represents the pairwise correlation between polymorphims (measured as D' (**a**) or r^2 (**b**)). The black to white gradient reflects higher to lower values. The plots were generated by HaploView 3.32.[30]

Table 1

Demographic and clinical characteristics of the patient population eligible for the pharmacogenomic study

Characteristic	*N (%)*
Total	144
Sex	
Male	82 (56.9)
Female	62 (43.0)
Age (Mean, range)	59 (25–82)
Primary tumor site	
Colon	111 (77)
Right	*39 (35)*
Left	*68 (61)*
Transverse	*3 (3)*
Sigma	*1 (1)*
Rectum	33 (23)
Stage of disease at diagnosis [a]	
II	21 (15)
III	123 (85)
Neo-adjuvant radiotherapy (only for rectum)	
Yes	19 (58)
No	14 (42)
Neo-adjuvant chemotherapy (only for rectum)	
5-FU	10 (30)
5-FU—FA and oxaliplatin	3 (9)
Number of cycles (Mean, range)	10.4 (1–12)
Patients who received all 12 planned cycles	81 (56)
Total (mg) oxaliplatin dose	
Median (range)	1460 (145–2166)
Dose per m^2 (mg m^{-2}): median (range)	886 (85–1020)
Dose per m^2 per week: median (range)	35 (17–54)

[a] TNM scale.

Table 2

Common toxicities in patients treated with FOLFOX4

Toxicity	*Patients*			
	Any grade No. of patients	*%*	*Grade 3–4 [a] No. of patients*	*%*
Non-hematological toxic effects				
Diarrhea	53	36.8	16	11.1
Nausea	68	47.2	3	2.1
Vomiting	30	20.8	4	2.8
Asthenia	44	30.5	0	0
Alopecia	19	13.2	0	0
Mucositis	32	22.2	2	1.4
Hepatic (hyperbilirubinemia)	6	4.2	1	0.7
Infection without severe neutropenia	9	6.2	0	0
Neurotoxicity[b]	120	83.3	10	6.9
Hematological toxic effects				
Anemia	64	44.4	0	0
Neutropenia	91	63.2	54	37.5
Leukopenia	54	37.5	8	5.5
Fever with severe neutropenia	4	2.8	2	1.4
Thrombocytopenia	59	41.0	1	0.7

[a] According to the NCI-CTC version 3.

[b] According to the oxaliplatin-specific scale (23).

Table 3

Associations between genetic polymorphisms previously related to toxicity in FOLFOX4 metastatic CRC

SNP	*Gene*	*Base change*	*MAF*		*Most significant genetic model*		
			Grade ≥2 toxicity	*Grade 0–1 toxicity*	*Model*	*OR (95% CI)*	P[a]
Neurological toxicity							
rs947894	*GSTP1*	A>G	0.321	0.301	Dominant	1.16 (0.59–2.30)	0.6647
rs1138272	*GSTP1*	C>T	0.071	0.080	Additive	0.91 (0.35–2.38)	0.8433
rs4426527	*AGXT*	A>G	0.241	0.247	Additive	0.93 (0.52–1.65)	0.7946
rs34116584	*AGXT*	C>T	0.277	0.227	Additive	1.29 (0.73–2.27)	0.3863
N/A	*AGXT*	*del-74bp*	0.232	0.276	Additive	0.73 (0.40–1.32)	0.3029
		Grade 0–2 toxicity					
Neutropenia	Grade ≥3 toxicity	Gene					
N/A	GSTM1	deletion	0.192	0.237	Additive	0.73 (0.41–1.32)	0.2995

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] Adjusted for sex, age, neo-adjuvant therapy and oxaliplatin cumulative dose normalized by BSA (for neurological toxicity).

Table 4

Significant associations between genetic polymorphisms and grade ≥2 neurological toxicity. Associations with q<0.10 are in bold

SNP	*Gene*	*Base change*	*MAF*		*Most significant genetic model*			
			Grade ≥2 toxicity	*Grade 0–1 toxicity*	*Model*	*OR (95% CI)*	P[a]	q**
rs2074087	*ABCC1*	G>C	0.0750	0.2529	Additive	0.43 (0.22–0.86)	0.0170	**0.0881**
rs35587	*ABCC1*	T>C	0.2455	0.3000	Dominant	0.47 (0.23–0.96)	0.0375	0.1049
rs1885301	*ABCC2*	G>A	0.5982	0.4419	Recessive	3.06 (1.35–6.92)	0.0072	**0.0747**
rs717620	*ABCC2*	C>T	0.2455	0.1782	Recessive	14.39 (1.63–127.02)	0.0164	**0.0881**
rs2273697	*ABCC2*	G>A	0.1340	0.2300	Dominant	0.44 (0.20–0.98)	0.0434	0.1049
rs4148396	*ABCC2*	C>T	0.4629	0.3512	Recessive	4.69 (1.60–13.74)	0.0048	**0.0747**
rs3740066	*ABCC2*	C>T	0.4545	0.3353	Recessive	2.99 (1.16–7.70)	0.0231	**0.0958**
rs2622604	*ABCG2*	C>T	0.3092	0.2118	Recessive	3.61 (1.01–12.88)	0.0478	0.1049

ABCC2 haplotype	*rs1885301*	*rs717620*	*rs2273697*	*rs4148396*	*rs3740066*	*Model*	*OR (95% CI)*	*P*	q**
(I) (Protective)	G	C	A	C	C	Additive	0.60 (0.30–1.16)	0.1296	—
(II) (Risk)	A	T	G	T	T	Additive	1.72 (0.95–3.09)	0.0722	—

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio.

** FDR-adjusted *P*-value.

[a] Adjusted for sex, age, neo-adjuvant therapy and oxaliplatin cumulative dose normalized by BSA.

Table 5

Significant associations between genetic polymorphisms and grade 3–4 toxicity. Associations with $q<0.10$ are in bold

SNP	*Gene*	*Base change*	*MAF*		*Most significant genetic model*			
			Grade ≥3 toxicity	*Grade 0–2 toxicity*	*Model*	*OR (95% CI)*	P[a]	q[**]
Neutropenia								
rs3136228	*hMSH6*	T>G	0.509	0.410	Recessive	3.23 (1.38–7.57)	0.0071	**0.0937**
rs35587	*ABCC1*	T>C	0.204	0.325	Additive	0.54 (0.31–0.96)	0.0368	0.1273
rs717620	*ABCC2*	C>T	0.269	0.165	Additive	1.81 (1.01–3.26)	0.0466	0.1273
Any non-hematological toxicity								
rs1799794	*XRCC3*	A>G	0.365	0.225	Recessive	8.90 (2.48–31.97)	0.0008	**0.0150**
rs861539	*XRCC3*	C>T	0.2917	0.4435	Additive	0.49 (0.24–1.00)	0.0495	0.1326
rs3213239	*XRCC1*	InDel	0.212	0.406	Additive	0.39 (0.19–0.82)	0.0130	0.1217
rs1130409	*APE 1*	T>G	0.6042	0.4396	Additive	1.99 (1.05–3.74)	0.0339	0.1326
rs1136410	*PARP*	T>C	0.2500	0.1710	Dominant	2.77 (1.07–7.21)	0.0366	0.1326
N/A	*GSTT1*	Gene deletion	0.500	0.364	Recessive	2.82 (1.02–7.83)	0.0467	0.1326

Abbreviations: CI, confidence interval; MAF, minor allele frequency; N/A, not applicable; OR, odds ratio.

[**] FDR-adjusted *P*-value.

[a] Adjusted for sex, age, neo-adjuvant therapy.